SOME FACTORS INFLUENCING NITROGEN FIXATION AND NITRIFICATION[1]

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Progress in the knowledge of soil bacteriological investigations must necessarily depend upon an acquaintance with the individual factors that determine the efficiency of a particular group of soil organisms. Once the relative importance of these factors is determined, the unessential discriminated from those which are of paramount importance, a constructive policy for the promotion of bacterial activity may be announced that will be reflected in an increased fertility of agricultural lands. Investigations on the two general processes, nitrogen fixation and nitrification, have been reported recently from these laboratories.[2] These reports have announced the effects of various organic and inorganic fertilizers in developing certain bacterial floras; the stimulus exerted by cultivation on the two processes just mentioned has been noted; and the comparison of a number of soil types with reference to their bacterial activities was emphasized in the investigations. The results of this work were such as to suggest a number of interesting possibilities to account for unusual conditions that were here and there encountered. Accordingly, an effort has been made to detect some of the influences that are responsible for these conditions and to place certain factors in the relative positions of importance. The data in this paper, which are in every case somewhat preliminary are closely connected with those of the previous publication and are accumulative toward establishing certain contentions which have heretofore been suggested.

The moisture content of a soil with relation to its free nitrogen fixing power is a matter which it would be well to investigate. The rather meager information on this subject has recently been aug-

[1] Paper 44 from Lab. Plant. Path. and Bact. Va. Agric. Exp. Sta.

[2] REED, H. S., and WILLIAMS, BRUCE, Nitrogen fixation and nitrification in various soil types. Va. Agric. Exper. Sta. Tech. Bull. 3. 1915.

mented by an interesting article by LIPMAN and SHARP,[3] giving the point at which maximum fixation is reached in a certain soil and suggesting that there are really two maxima of fixation with reference to moisture content, the one that is most favorable to aerobic bacteria, and the other at which the anaerobic forms most actively flourish. These tests measured nitrogen fixation under the influence of varying percentages of moisture. Of significance also is the effect of various periods of drying on the vitality of the nitrogen fixing flora. The ability of those organisms to withstand long exposure to drying serves somewhat as an index to their activity in arid regions and in more temperate climates after long periods of drought.

It is true also that if any progress is ever to be made in attempts to introduce cultures of free nitrogen fixing organisms, *Azotobacter*, for instance, artificially into soils, the persistence of such species under exposure to drying will be an important determinant in their efficiency. Such phases of the problem as these received attention in the present tests.

Soils which had been in the laboratory 12–18 months, inclosed in glass jars and practically air dried, were employed. All of these soils had been tested originally for this nitrogen fixing power, giving additions of nitrogen ranging from 2.3 to 16.7 mg. per 1 gm. of mannite consumed. *Azotobacter* had been isolated and grown in pure culture from probably 25 of the soils. All of these soils were now taken and 5 gm. inoculations were made into 25 cc. of Ashby's medium in order that qualitative examination might be made for the organism. Examinations made after 4 days revealed *Azotobacter* in only 4 of the soils. Attempts to isolate the organisms from these were unsuccessful, although it is probable that if the efforts had been repeated the organism would finally have been obtained. A number of soils from which it was positively known that *Azotobacter* had been isolated when first brought to the laboratory were then taken, their moisture content brought up to 20 per cent, and the soils kept in the incubator room at 28° C. for 3 weeks. An opportunity was then afforded for the bacterial flora to revivify

[3] LIPMAN, C. B., and SHARP, L. T., Effect of moisture content of a sandy soil on its nitrogen fixing power. BOT. GAZ. **59**:402–406. 1915.

itself and regain something of its original status, but similar qualitative tests at the end of 3 weeks showed *Azotobacter* present in only 3 soils, and from one alone was the organism successfully isolated. It appeared from these tests that one at least of the free nitrogen fixing organisms had materially deteriorated through the processes of drying attendant upon 10 months' storage of the soil in the laboratory.

To determine to just what extent drying affected the fixing power of the soils as a whole was the object of the next series of tests. Four soils were selected which had exhibited rather extraordinary fixing powers when first brought from the field, but which had been in the laboratory for 15 months. Fixation tests were made with them, the solution method being employed, which uses 10 gm. of soil in 100 cc. of Ashby's medium, incubated for 21 days, with total nitrogen analysis for increase at the end of that period. The films which formed on two of the flasks were good and gave evidence of the presence of a vigorous culture of *Azotobacter*, but the two remaining ones did not exhibit this characteristic evidence. In table I the efficiency of the soils in question for fixing nitrogen in solution is given.

TABLE I

EFFECT OF DRYING ON THE NITROGEN FIXING POWER OF CERTAIN SOILS

No.	Moisture content after 15 months	Mg. nitrogen fixed per 100 cc. Ashby's medium		
		November 1913	January 1915	Percentage of decrease
1.....	1.8	10.6	7.5	30
2.....	1.3	13.4	8.1	40
3.....	1.0	15.4	11.6	24
4.....	3.8	13.9	7.8	43

The soils lost 24–43 per cent of their original efficiency for fixing nitrogen during the period of 15 months' storage. Whether this falling off in nitrogen fixing power is due particularly to attenuation of aerobic or anaerobic forms is difficult to say, but from evidence previously obtained on the persistence of *Azotobacter* under drying, as previously noted, it is probable that the vitality of this

organism was materially lowered, and to this condition may be referred in large measure the decrease in addition of nitrogen.

A rich garden soil was obtained in the spring of 1915. It was known to have a vigorous *Azotobacter* flora and was examined to determine the effect of short periods of drying on its fixing power. Tests were made at various intervals up to 30 days. The results are recorded in table II.

TABLE II

THE EFFECT OF SHORT PERIODS OF DRYING ON NITROGEN FIXING POWER OF GARDEN SOIL

Percentage of moisture	Date of tests	Mg. nitrogen added by 10 gm. soil in 100 cc. Ashby's medium (after 21 days)
9.1.....	June 7	16.4
6.3.....	" 8	17.0
2.9.....	" 9	14.8
1.5.....	" 18	10.1
1.2.....	July 7	10.1

The gradual decrease in the moisture content of the soil was not reflected in any notable falling off in its nitrogen fixing power until the test made after 10 days' drying. After 30 days, the moisture content remaining stationary, there was no further diminution. Up to this time the nitrogen fixing flora had retained practically 60 per cent of its original efficiency for fixing nitrogen.

The nitrogen fixing flora significantly decreases in its activity under the influence of drying, as the foregoing tests indicate. This deterioration is manifested as early as two weeks after removing the soil from the field and exposing it to the ordinary laboratory conditions of drying. After 15 months, however, a number of soils retained an ability to fix nitrogen that was somewhat surprising, which indicates that some species at least have considerable resistance to drying. A number of qualitative tests for *Azotobacter* in soils kept in storage for 15 months indicate that this organism is more easily attenuated than some other species.

The striking failure of some soils to fix nitrogen lends itself to speculations as to the underlying causes for such a condition. It appears, however, that the nitrogen fixing flora is so intimately

connected with the humus content of a soil that this latter factor is by far the most important influence in connection with the process. The possibility that certain other factors lend a depressing effect has been eliminated to a degree from consideration in this laboratory by results of certain work with reference to toxic conditions of the soil. Whatever may be the inimical effects on higher plants of a toxic condition, if such in reality often exists, it is extremely doubtful whether similar effects are exerted on the bacterial flora. At least a number of compounds which are supposed to be responsible for soil toxicity have been shown to exhibit little deleterious effect on the growth of *Azotobacter*.[4] Evidence from further tests goes to show that the soil extract itself probably does not carry substances which would retard fixation.

Three soils which possessed conspicuously low fixation powers were selected and extracts made therefrom. The extracts were made into Ashby's medium and fixation tests with pure cultures of *Azotobacter* were carried out. Along with the flasks containing the extracts were controls using Ashby's solution made with distilled water. The same strain of pure culture was used for inoculating all the flasks. After 21 days the contents of the flasks were analyzed for total nitrogen. The nitrogen fixed by the culture grown on the extracts from the 3 soils was 6.5, 7.6, and 6.7 mg. nitrogen respectively. Those grown on the control flasks gave 6.8 mg. There was certainly no depressing effect registered here from using extract from soils deficient in nitrogen fixing power in substitution for distilled water in the culture medium.

The studies on nitrification (*loc. cit.*), which up to this time had included an examination of some 93 soils, were continued along with the work on fixation. Among the soils examined were a number which had exhibited signal evidence of poor nitrifying power. These in the majority of cases would be classed as poor soils from a practical agricultural standpoint, and their bacterial activity was closely correlated with this condition. Previous tests with lime had emphasized the stimulus which it exerts on nitrification, and to measure its efficiency on these soils, particularly deficient

[4] REED, H. S., and WILLIAMS, BRUCE, The effect of certain organic soil constituents upon nitrogen fixation by *Azotobacter*. Va. Agric. Exper. Sta. Tech. Bull. 4. 1915.

in their nitrifying power, would be a matter of considerable interest. Since it is likely that the humus content of a soil most intimately of all factors controls its nitrifying efficiency, it was decided to observe the effect of the addition of sugar to these soils in comparison with that produced by the addition of lime. Accordingly, two 100 gm. portions of each soil were measured out into glass jars, the moisture content brought up to 20 per cent, and the jar placed in the incubator room. To one portion of the soil 0.15 per cent $CaCo_3$ was added, and to the other 2 per cent of mannite. After 3 weeks, a period allowed for the lime and sugar to exert some influence on the bacterial flora, an optimum moisture content being maintained, 0.1 per cent ammonium sulphate was added to measure the nitrifying power of the soils. All of these soils, it will be recalled, had been tested for nitrifying power without the addition of lime, and a threefold comparison was thus afforded. Table III gives the quantities of nitrates found with and without lime.

TABLE III

COMPARATIVE NITRIFYING POWER OF CERTAIN SOILS WITH AND WITHOUT LIME

No.	MG. OF NITRATE NITROGEN PER 100 GM. SOIL		No.	MG. OF NITRATE NITROGEN PER 100 GM. SOIL	
	Without $CaCO_3$	With 0.15 per cent $CaCO_3$		Without $CaCO_3$	With 0.15 per cent $CaCO_3$
1	.....	.....	13	.....	2.12
2	.....	1.5	14	0.6	7.6
3	.....	.....	15	1.8	7.2
4	.....	.....	16	.....	1.4
5	3.3	8.8	17	1.4	8.8
6	5.1	1.2	18	.....	1
7	3.0	.....	19	1	1
8	7.2	12	20	3	5
9	.....	.....	21	1	1
10	6.5	1.6	22	.....	Trace
11	.....	2.2	23	1	1
12	.....	1			

Without the use of lime a number of soils completely failed to nitrify ammonium sulphate, and few exhibited what might be termed an average nitrifying power as judged by the method employed. While under the influence of lime there is an increase in the majority of the soils, although the stimulation is not especially

noteworthy. It appears that where there is some development of the nitrifying flora in the soil originally the effects of lime are decidedly more evident than in those soils apparently devoid of nitrifying power. The results of these tests are not to be construed as evidence depreciating the effect of lime on nitrification. They are contributory to the fact that the nitrifying flora of a soil cannot be developed merely by the use of lime in the absence of other factors more fundamental in their influence than is lime itself.

There was no formation of nitrates whatever under the influence of 2 per cent mannite. It not only failed to afford a source of energy for the nitrifying ferments, but exerted a depressing effect on the activities of such as were present. Smaller quantities of various sugars (0.5 per cent) have in some instances exerted a beneficial effect on nitrification.[5] The quantity used in these tests was possibly large enough to have a toxic effect on the soil organisms. It seems well established, however, that a satisfactory pabulum for nitrifying organisms cannot thus be so readily established.

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[5] COLEMAN, L. C., Untersuchungen über nitrifikation. Centralbl. Bakt. 20^2:401. 1908.